C: Furlow

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MAR 8 1991

MEMORANDUM

SUBJECT: Methyl Bromide Industry Panel: Response to the Methyl Bromide Reregistration Standard: Metabolism Studies (MRID # 41627701, DEB # 7101.).

FROM: R. B. Perfetti, Ph.D., Chemist
Reregistration Section
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RB Perfetti

THRU: W. J. Boodee, Section Head
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RBP for

TO: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

and

L. Rossi, Chief
Reregistration Branch
Special Review and Reregistration Division (H7508C)

Attached is a review of methyl bromide (MeBr) metabolism studiesy submitted by the Methyl Bromide Industry Panel in response to the MeBr Reregistration Standard. These studies were reviewed by Acurex Corporation under supervision of CBRS, HED.

These studies have undergone secondary review in CBRS and have been revised to reflect the Branch policies.

Please see our conclusions in the attachment regarding the adequacy of the information provided by the Registrant.

If you need additional input please advise.

Attachment 1 : Review of the Methyl Bromide Metabolism Studies.

cc: With Attachment 1: R. B. Perfetti, J. Burrell (PIB/FOD), Methyl Bromide Reregistration Standard File, Methyl Bromide Subject File, C. Furlow (PIB/FOD), Dynamac, Circ. (7).

cc: Without Attachment: P. Fenner-Crisp (HED) and RF.

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METHYL BROMIDE
(DEB No. 7101)

TASK 3

**Registrant's Response
to Residue Chemistry Data
Requirements**

January 28, 1991

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

Submitted by:

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METHYL BROMIDE (DEB NO. 7101)

REGISTRANT 'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task-3

BACKGROUND

The Methyl Bromide Guidance Document dated 8/86 requires data concerning the nature of the terminal residues of methyl bromide in plants fumigated post-harvest. Additionally, the Guidance Document states that pre-plant fumigation studies may be required if post-harvest studies reveal metabolites of concern other than methyl bromide per se (MeBr per se) and inorganic bromide ion (iBr), and animal studies may be required if residues of concern are found in animal feed raw agricultural commodities (RAC). The Guidance Document has specific requirements for the analysis of MeBr per se, iBr, and methylated and brominated derivatives of natural plant constituents, particularly such products as purine and pyrimidine bases.

Additional interpretation of requirements set forth on the nature of the residue in plants may be found in a series of DEB memoranda. In a memo dated 11/3/88 (RCB No. 4399), by C. Deyrup, six important points were brought up. First, the ion selective electrode method for inorganic bromide (iBr) was found inappropriate due to high variability. Second, it was agreed that the presence of 5-bromouracil could be checked for chromatographically. Third, a requirement to account for the contribution of volatiles to total radioactive residue (TRR) was explicitly stated. Fourth, the need to include bruised and stemless fruit in all tests was stated. Fifth, residue data on grain dust is required. Finally, data on waxed vs. unwaxed commodities is required.

In a memo dated 12/8/88, C. Deyrup emphasized the need to specify fumigation temperatures in the required tests. In addition, the memo stated that a fumigation load factor "<10%" should be used to represent the worst practical commercial case.

In a third memo dated 2/9/89 (DEB No. 4680), the same reviewer judged as adequate (with some modifications) the Methyl Bromide Industry Panel (MBIP) study plan. It stated that the formation of methylated glutathione should be investigated along with S-methyl cysteine as a precursor to methyl mercaptan. It stated that MeBr per se is the only volatile residue which needs to be determined and that it would be permissible to generate data using non-radioactive material if the fumigation technique showed good precision. This would involve determining levels of MeBr after a 1-to-2 hour aeration period since this is typical commercial practice. It also specified that TRR would be estimated as MeBr per se plus chemically bound residues if the scheme in the second modification was followed. Finally, it required that all ether extracts be included in the TRR. A memo dated 4/25/89 (DEB 5001), by C. Deyrup, re-emphasized the specific wording of the previously described memo.

A preliminary study by MBIP was reviewed by N. Dodd (DEB No. 5834) who concluded that methyl glutathione may be formed; that S-methyl cysteine is a possible source of methyl mercaptan; and that S-methylmethionesulfonium salts are a possible source of dimethyl sulfide. The memo cited the specific activity of the [^{14}C]MeBr as used in the studies and cited the temperature during fumigation as "20-23 °C."

The final report submitted by the MBIP (MRID 41627701) contains data pertaining to the nature of the residue in commodities fumigated post-harvest with MeBr. These data are reviewed here for their adequacy in fulfilling outstanding residue chemistry data requirements.

CONCLUSIONS

1. The qualitative nature of the residue in plants is not adequately understood. This determination is based primarily upon the failure of the registrant to demonstrate mass-balance of the total residue between physically and chemically bound forms. The TRR data Table 3 of the current submission results from a different exposure than was used for the characterization study. Thus, these data are not transferable. Information is not presented to permit calculation of % ^{14}C characterized.

2. Chemically bound residues are primarily methylated proteins. Evidence of O-, S-, and N-methylated species was found. Specifically identified residues include MeBr, S-methylcysteine, 1-methylhistidine, 3-methylhistidine, 7-methylguanine, 1-methyladenine, 3-methyladenine, 3-methylcytosine, 3-methylguanine, and S-methylglutathione. The failure to provide valid total residue data makes it impossible to calculate concentrations for the identified residues.

3. 5-Bromouracil was tested for and was not detected. However, it should be noted that other mutagenic or potentially mutagenic brominated and methylated bases were not specifically quantified as part of this study. A printout from Oak Ridge laboratories of potential residues of concern was provided to RD by CBRS. In the discussions between MBIP and the Agency, 5-Bromouracil was used as an example and <u>not</u> as the only residue of concern.

4. Determination of inorganic bromide remains as a data gap since it was not conducted as part of this work. While the importance of iBr is currently open to argument, iBr remains a data requirement of the Guidance Document.

5. There is no evidence that proportionate quantities of bruised and stemless fruit were included.

6. No data were presented to verify the required fumigation load factor of <10%. This remains a concern since the load factor will affect the detectability of residues of concern. 5-Bromouracil is an example.

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7. No data were presented to describe the nature of the residue for grain dust. Grain dust is of concern as a potential animal feed. It should be noted that the grain may be sprayed with food grade mineral oil to minimize grain dust in the air. This mineral oil may be expected to modify the levels of free MeBr residues, in particular, and all residue levels in general.

8. No data were presented for a comparison between waxed and unwaxed fruit.

9. No raw data or example calculations were provided.

RECOMMENDATIONS

The registrant should provide evidence that proportionate numbers of bruised or stemless fruit, as per memo of 11/3/88 (DEB 4399), were used in the studies reported as part of MRID 41627701. If the registrant cannot provide such evidence, experiments intended to quantify physically and chemically bound residues should be repeated.

The fumigation load factors estimated from the section entitled "Methyl bromide treatments " (pp. 18-20 of the submission) do not appear to satisfy the required <10% as specified in the memo dated 12/8/88. The registrant should provide evidence that this requirement was satisfied. If the registrant cannot provide such evidence, experiments intended to quantify physically and chemically bound residues should be repeated. Load factor remains a concern during nature of the residue studies since it may affect the detectability of low level residues of concern (5-Bromouracil is an example).

The requirement for data on grain dust remains outstanding. Grain dust is of concern as a potential animal feed. It should be noted that the grain may be sprayed with food grade mineral oil to minimize grain dust in the air. This mineral oil may be expected to modify the levels of free MeBr residues, in particular, and all residue levels in general. The registrant should provide the required information.

The requirement for a comparison of waxed vs. unwaxed fruit remains outstanding. The registrant should provide the required information.

The registrant should resolve inconsistencies from the submission. An example may be found on p. 19 of the submission (MRID 41627701) which states, "At the end of treatment, commodities were aerated overnight or longer before further handling. " This is at odds with Tables 2 and 3 of the submission which show 15-minute aeration data, and with Table 4 of the submission which shows 1-hour aeration data (p. 35 of the submission, as part of the Results and Discussion section). In addition, Table 4 provides data appropriate to the estimate of TRR. However, it is not stated in the submission that the statistical data provided are from replicate fumigation experiments, as required

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by the C. Deyrup memos of 2/9/89 and 4/25/89 (DEB Nos. 4680 and 5001, respectively).

Raw data and example calculations are not provided in the submission. This could be corrected during a rewrite.

Animal metabolism studies should be required. This recommendation is based upon the high level (>100 ppm) of physically bound MeBr found in alfalfa and high oil level animal feeds. Upon ingestion, MeBr would be available to methylate protein and DNA.

TRR experiments should be redone since there is no evidence for equivalence of physically and chemically bound experiments. While these experiments were done at the same concentration of MeBr and exposure period, it is not clear that such variables as load factor were identical between the two sets of experiments, as is required for comparability of data. Although the registrant included calculated TRR data (Table 25 in submission), there is no evidence that the physically bound residues result from replicate fumigations, as was required. Data for physically bound residues from replicate fumigations is necessary to demonstrate that the minimal standards for data variability needed to justify the calculation of TRR as the sum of unlabelled MeBr and labelled bound residue results were attained. The TRR data presented in Table 3 of the submission results from preliminary experiments at 24mg [^{14}C]MeBr/L for 24 hours (1x) exposure level while characterization studies were performed at 48mg [^{14}C]MeBr/L for 72 hours (<2x). Thus these data are not transferable and do not permit calculation of percent characterization. Additionally, there is no evidence that fumigator load factors were <10%, nor that a proportionate quantity of bruised and stemless fruit were used. Finally, it should be noted that methanol was not determined as a potential volatile residue. Since no data has been presented which permits determination of total residues, it is not clear whether or not methanol residues are a significant fraction.

DETAILED CONSIDERATIONS

Treatment with MeBr

Maize, wheat, oatmeal, almonds, peanuts, alfalfa, oranges, apples, potatoes, calf thymus DNA, and salmon testes DNA were fumigated in bench-scale fumigators. These fumigators were either desiccators fitted with septa or flasks fitted with adapters and septa.

An aliquot of the commodity (by weight or unit) was placed in a fumigator. A mixture of labeled and unlabeled MeBr was added by syringe. In a typical experiment, the MeBr concentration was on the order of 48 mg/L (~2x), and fumigation exposure occurred for 3 days at a temperature of 20-23 °C. The actual, initial MeBr concentration was determined by GC/FID using a 2 meter nickel column packed with 120-140 mesh Chromosorb 102. These conditions represent fumigation at elevated levels (~2x). This was done intentionally to improve the detectability of any significant metabolites.

A potential error in this fumigation technique would be losses due to leakage or adsorption onto some surface within the fumigator. One experiment was performed, in duplicate, in which a fumigator contained a concentration of MeBr without any commodity for a period of 72 hours. The initial concentration was measured at 24 mg/L. The mean final concentration was 23.2 mg/L. This represented a loss of 3%. In a parallel experiment with commodity present (wheat), the initial concentration was 48 mg/L and the final concentration was 25.4 mg/L.

Total Radioactive Residues (TRR)

Per C. Deyrup memos dated 2/9/89 and 4/25/89, TRR may be estimated as the sum of the physically bound MeBr per se and the chemically bound residues provided that certain conditions are met. These include a satisfactory level of precision for the MeBr analysis of replicate fumigation trials and that chemically bound residues be determined from equivalent MeBr treatment and aeration. Table 1 presents a calculated TRR, based upon data from Tables 4 and 7 of the submission (MRID 41627701). However, it is not clear as to whether or not the requirements for equivalence and replicate fumigation trials were met. The final column of data in Table 1 adjusts TRR to a 1x equivalent.

Careful examination of MRID 41627701 suggests that equivalence of sample age is not met for some samples. Footnote c of Table 13 and footnote b of Table 14, of the submission (MRID 41627701), state that fumigated maize and almond samples were stored at room temperature for a period of 6 months before any further processing occurred. Additionally, p. 19 of the submission states that commodities were aerated for a period of "overnight or longer " before any further sample manipulation occurred. Based upon these observations, the requirements for estimation of TRR and

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apportionment of physically and chemically bound residues are not satisfied. Since data are not available for total residues it is not possible to determine the % characterized (equivalent to % ^{14}C characterized in most studies). It is not possible to calculate the concentration of residues as a percent of total residues.

The issue of equivalent samples is important to estimating the TRR as is shown by data in Table 22 of the submission. In this table, almonds and maize samples are compared in terms of analysis "immediately after fumigation" and after 6 months storage at room temperature before sample preparation. Almonds analyzed in November showed 470,047 dpm/g, while almonds analyzed and prepared in May after 6 months of storage showed 623,087 dpm/g. The implication of these numbers is that residual, physically bound MeBr continues to react during storage. It should be noted that the submittors suggest a different explanation; i.e., changes in moisture content may have accounted for a 33% increase in concentration. Data on moisture content were not provided.

Physically Bound Residues

Following treatment with MeBr, commodities were aerated in open containers at 18-24 °C. This was performed for each commodity in a fume hood with a measured face velocity of 0.76 m/sec for variable periods of time to generate decline curve samples.

MeBr per se was determined in fumigated commodities by a modification of the King headspace procedure. These modifications are described in the appendix to MRID 41627701 and are intended to minimize a potential problem of water droplet blockage.

A weighed aliquot of the fumigated commodity was homogenized in a high-speed Waring blender with a specified volume of water. The MeBr partitions between the aqueous and gas phases during a period of 17 min in a 26 °C water bath. Analysis is performed by GC/FID on an aliquot of the headspace. The GC was equipped with a 2-meter column packed with 60-80 mesh Tenax GC at an isothermal oven temperature of 105 °C.

The analysis by GC/FID used in this work does not offer specificity to MeBr. It is reported that preliminary studies were performed using a GC/ECD. However, the levels of analyte were at elevated levels that saturated the ECD detector.

Table 1 presents data for the estimation of TRR at the 1-hour aeration time. This time period simulates that used in commercial practices. No data was provided to show whether or not replicate fumigations were performed. As a consequence, it cannot be determined what, if any, amount of variation there was between duplicate samples. This is important since permission to estimate TRR as a summation of physically and chemically bound residues was given only "If there is little variation in residue levels from replicate fumigations, ..." The requirements for minimal variation were not addressed.

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Table 2 represents data for physically bound MeBr and the decline of MeBr residues with aeration time.

Chemically Bound Residues

Extraction of Residues

Fats and oils were removed from treated commodities after homogenization by soxhlet extraction using diethyl ether. Defatted meals were stored at 4 °C. Fats and oils were recovered by removal of the diethyl ether solvent using a rotary evaporator.

Hydrolysis of Residues

A mixture of MeBr-treated commodity and methyl methioninesulfonium chloride was heated and refluxed with 1 N NaOH solution for 5 hours. A slow stream of nitrogen gas was used to carry vaporized products through a side arm to a series of traps. Trap 1 contained chilled water. Traps 2 and 3 contained saturated aqueous solutions of mercuric cyanide. Traps 4 and 5 contained saturated aqueous solutions of mercuric chloride. The contents of the traps were collected and analyzed for radioactivity by liquid scintillation spectroscopy (LSS). The results of this analysis may be found in Table 3. Excellent recoveries, in the range of 90-111%, were observed for the radioactivity using LSS.

Separate quality control tests confirmed that methyl mercaptan was collected in trap 3 and that dimethyl sulfide was collected in trap 5. Methanol was confirmed in trap 1 by derivatization with 3,5-dinitrobenzoyl chloride and recrystallization of the product.

The basic solutions resulting from the above process were neutralized with concentrated hydrochloric acid and concentrated by lyophilization. Residues were hydrolyzed by heating and refluxing in 6 N hydrochloric acid, in the presence of inert nitrogen gases, for 20-24 hours. The water soluble product was fractionated by ion-exchange chromatography.

DNA was isolated from MeBr treated maize and analyzed for radioactivity. Isolation was performed by extraction of defatted meal with 1% cetyltrimethylammonium bromide buffer and centrifugation. The supernatant was extracted with chloroform:isoamyl alcohol (24:1). The isolated DNA was hydrolyzed with formic acid. The formic acid was removed in vacuo and the bases were redissolved in 0.1 N hydrochloric acid solution. Samples were chromatographed on three separate systems.

Fractionation of Acid Hydrolysates

The water soluble product of the acid hydrolysis was applied to a Dowex 50W-X8 column in the protonated (H^+) form. The column was eluted successively with 300 mL

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of water, 700 mL of 2 N pyridine, and 700 mL of 3 N ammonium hydroxide. Twenty-five mL fractions were collected and analyzed for radioactivity by LSS. Three peaks were detected. Fractions corresponding to a detected peak were combined and concentrated to dryness by rotary evaporation.

Material from the third detected peak, which eluted with ammonium hydroxide, was dissolved in water, acidified with 6 N HCl and concentrated to dryness by rotary evaporation. This material was dissolved in water and applied to a Dowex 50W-X8 column in its ammoniated (NH_4^+) form. The column was eluted using the same scheme as above. Two peaks were detected by LSS. The results of these analyses may be found in Table 4.

Material collected from peak 2 in the H^+ form and from peak 1 in the NH_4^+ form of Dowex 50W-X8 was analyzed for 1- and 3-methylhistidines by HPLC using a µBondapak C_{18} semipreparative column and a Partisil 10 SCX column.

Characterization of Residues

Methylation of proteins and amino acids

The results of the 1 N NaOH hydrolysis step presented in Table 3 clearly confirm the trapping of volatile radioactive products. The contents of trap 1 were identified as methanol by derivatization with 3,5-dinitrobenzoyl chloride. The authors point out that any methanol due to the hydrolysis of MeBr per se would be lost during earlier sample preparation steps. The implication of this is that methanol trapped at this stage must result from O-methylated amino acids.

Quality control experiments were conducted to confirm that methyl mercaptan is stable to the 1 N NaOH treatment and that it is trapped by the mercuric cyanide solutions. While no experiments were performed to define the exact source of the methyl mercaptan generated from the treatment of fumigated commodities, the authors suggest S-methyl cysteine as the logical source. Separate tests did confirm that similar results are obtained by treating S-methyl cysteine in a manner identical to the samples. Additionally, when three commodities, maize, potatoes, and oranges, were extracted with 60% ethanol and subjected to ion exchange chromatography, as described above, the peaks observed corresponded in retention volume to S-methyl cysteine and S-methyl glutathione. The peak equivalent to S-methyl cysteine was rechromatographed by HPLC on a µBondapak C_{18} column. The peak resulting from this process and authentic S-methyl cysteine were derivatized with 1-fluoro-2,4-dinitrobenzene and chromatographed again with equivalent retention times.

The authors also suggest that the dimethyl sulfide observed is produced from methylated methionine.

Analysis of synthetic samples revealed that the material eluting as peak 2 from the H^+ form of the Dowex 50W-X8 has a retention time equivalent to 3-methylhistidine. Peak 1 of the NH^+ form of Dowex 50W-X8 corresponds in retention time to 1-methylhistidine and peak 2 corresponds to 1,3-dimethylhistidine.

DNA was isolated from MeBr-treated maize and analyzed for radioactivity. It was detected at the level of 0.34% of the bound (presumably, chemically) radioactivity. This is equivalent to 0.2 μgMeBr/g. Five methylated bases were observed in maize and almonds. These are 7-methylguanine, 1-methyladenine, 3-methyladenine, 3-methylcytosine, and 3-methylguanine, in approximate order of importance. Quantification of these products was not performed. Experiments were also performed which showed that radioactivity determined in the isolated DNA is a direct function of exposure time to [^{14}C]MeBr.

Glutathione

The presence of methylated glutathione is inferred from Table 15 of the submission which presents an inverse relationship between glutathione in MeBr-treated potatoes and the initial concentration of MeBr in the fumigator. The researchers propose that MeBr is metabolized in certain commodities by glutathione S-transferase-catalyzed conjugation. They state that "Peptidases would then hydrolyze the S-methyl glutathione to γ-glutamyl-S-methylcysteine and S-methylcysteine, substances which occur naturally in a variety of plants, including a number used for human consumption." In separate experiments, both S-methyl glutathione and γ-glutamyl-S-methylcysteine were observed. The registrant suggests that low moisture content commodities are not likely to exhibit this behavior.

5-Bromouracil

Isolated nucleic acids were hydrolyzed with a mixture of trifluoroacetic acid and formic acid. The reagent was removed by rotary evaporation and the product was dissolved in water. Separation of any 5-bromouracil was accomplished by sequential use of three HPLC systems. The appropriate fraction was collected and derivatized with a mixture of N,N-dimethylformamide and methyl iodide. The product was analyzed for the presence of 1,3-dimethyl-5-bromouracil by GC/ECD. Both untreated and MeBr treated wheat were carried through this protocol with indistinguishable results. Untreated wheat resulted in values of 0-131 pg of 1,3-dimethyl-5-bromouracil/g wheat, while treated wheat resulted in values of 0-94 pg of 1,3-dimethyl-5-bromouracil/g wheat. By contrast, wheat spiked with 5-bromouracil and carried through this protocol was clearly detectable.

SUMMARY

The data presented in this submission clearly demonstrate that the residues present after post-harvest fumigation are both physically bound MeBr and chemically bound residues.

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These chemically bound residues are primarily methylated proteins. Evidence of O-, S-, and N-methylated species was found. The relative importance of various sites of reactivity is a function of the commodity fumigated. Specifically identified residues include MeBr, S-methylcysteine, 1-methylhistidine, 3-methylhistidine, 7-methylguanine, 1-methyladenine, 3-methyladenine, 3-methylcytosine, 3-methylguanine, and S-methylglutathione. 5-Bromouracil was tested for, and found to be non-detectable. Inorganic bromide was not included in these studies. Evidence is presented that physically bound MeBr decreases with aeration time. The TRR data are judged to be not satisfactory since there is no evidence for equivalence of samples used for physically and chemically bound residue analysis. However, the registrant has failed to adequately characterize the residues from post-harvest fumigation in the following respects. Total residues have not been quantified either by determination of total radioactive residues (LSS determination of $^{14}CO_2$ generated by combustion of MeBr exposed RAC at time t=1hr post fumigation) or by demonstrating that the physically bound MeBr data are suitable for summation with the chemically bound residues. The registrant did provide data from LSS studies in their Table 3. This data resulted from exposure to 24mg [^{14}C]MeBr/L for 24 hours whereas characterization studies resulted from exposure to 48mg[^{14}C]MeBr/L for 72 hours. The data are not comparable. Lacking total residue data it is not possible to determine what percentage of the residues have been characterized by this work. The volatile residue methanol was not characterized as part of this work. Methanol may originate from the hydrolysis of MeBr. Since methanol has a lower vapor pressure than MeBr, which has detectable residues after aeration and storage, a gap may remain in the percent characterized unless methanol is also determined.

TABLE 1. ESTIMATE OF TRR
MeBr Equivalents (ug/g RAC)

RAC	Physically Bound	Chemically Bound	TRR
Maize	34.8	58.5	93.3
Potato	72.5	91.4	163.9
Alfalfa	115.7	262.4	378.1
Peanuts	107.6	149.7	257.3
Almonds	141.2	191.2	332.4
Oatmeal	4.1	366.6	370.7
Apples	82.0	7.7	89.7
Oranges [a]	54.5	39.5	94.0
Wheat	5.7	94.1	99.8

[a] Orange value is weighted average of peel and pulp data

TABLE 2. PHYSICALLY BOUND RESIDUES
MeBr(ug/g RAC)

RAC	Aeration Period(days) 0.042	1	2	4	10
Maize	34.8	9.6	4.4	1.5	0.09
SD[a]	9.8	1.3	0.8	0.6	0.05
%RSD[b]	28.0	14.0	18.0	40.0	56.0
Potato	72.5	17.6	11.0	2.7	0.18
SD	7.0	8.3	7.2	3.3	0.28
%RSD	10.0	47.0	65.0	122.0	156.0
Alfalfa	115.7	28.1	10.5	1.8	0.1
SD	c	4.5	1.2	2.1	0.08
%RSD	c	16.0	11.0	117.0	80.0
Peanuts	107.6	54.6	27.0	8.4	0.6
SD	5.8	1.8	2.3	0.8	0.06
%RSD	5.0	3.0	9.0	10.0	10.0
Almonds	141.2	90.4	59.1	44.1	13.5
SD	4.5	2.0	3.4	4.6	2.8
%RSD	3.0	2.0	6.0	10.0	21.0
Oatmeal	4.1	0.78	0.47	0.11	0.02
SD	1.6	0.25	0.11	0.03	0.01
%RSD	39.0	32.0	23.0	27.0	50.0
Apples	82.0	30.0	9.6	2.5	c
SD	28.4	4.6	2.8	1.5	c
%RSD	35.0	15.0	29.0	60.0	c
Oranges	54.5	8.9	2.5	0.5	c
SD	7.8	2.3	0.8	0.27	c
%RSD	14.0	26.0	32.0	54.0	c
Wheat	5.7	0.89	0.66	0.14	0.14
SD	1.56	0.49	0.51	0.035	0.11
%RSD	27.0	55.0	77.0	25.0	79.0

[a] SD = $\Sigma(X_i-\bar{X})/(n-1)$
[b] % RSD = 100 * SD/mean
[c] data not available

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TABLE 3. SPECIATION OF CHEMICALLY BOUND RESIDUES
MeBr Equivalents (ug/g RAC)

RAC	Chemically Bound	Trap 1 (MeOH) [b]	Trap 2 (MeSH) [c]	Total Trap 4 (Me2S) [d]	Trapped Volatiles	Non-Volatile Residue	% Recovery
Maize	58.5	17.1	2.3	13.2	33.0	20.2	91.0
Potato	91.4	9.1	15.3	25.2	51.0	34.0	93.0
Alfalfa	262.4	134.5	8.5	16.1	160.5	77.7	90.8
Peanuts	149.7	32.3	16.0	36.1	85.4	59.5	96.8
Almonds	191.2	30.1	9.4	22.2	62.6	132.7	102.1
Oatmeal	366.6	67.1	19.8	70.9	160.4	186.4	94.6
Apples	7.7	1.9	1.6	2.4	6.0	2.6	110.8
Oranges [a]	39.5	8.6	10.0	9.72	8.8	10.19	8.5
Wheat	94.1	20.1	4.1	27.3	52.7	37.1	95.5

[a] Orange values are weighted average of peel and pulp data
[b] Methanol
[c] Methyl mercaptan
[d] Dimethyl sulfide

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TABLE 4. SPECIATION OF CHEMICALLY BOUND RESIDUES
MeBr Equivalents (ug/g RAC)

RAC	Non-Volatile Residue	HCl Hydrolysate	% Recovery	Dowex 50W, Acid form Peak 1	Peak 2	Peak 3	% Recovery	Dowex 50W, Basic Form Peak 1	Peak 2	Recovery
Maize	20.2	18.1	90	4.1	4.5	6.8	85	5.5	1.1	96
Potato	34.0	28.4	84	21.0	1.1	1.8	84	0.9	0.3	68
Alfalfa	77.7	80.2	103	30.0	10.8	20.2	76	12.9	6.2	94
Peanuts	59.5	58.1	98	7.4	11.0	36.3	94	28.4	5.8	94
Almonds	132.7	146.1	110	11.7	23.5	110.2	99	98.4	8.3	97
Oatmeal	186.4	164.1	88	27.5	20.0	100.8	90	30.4	60.9	91
Apples	2.6	2.1	82	1.0	0.2	0.4	75	0.4	0.0	100
Oranges [a]	10.1	8.6	85	5.7	0.5	1.4	88	0.4	0.7	77
Wheat	37.1	28.3	76	7.7	6.6	14.5	102	9.3	5.0	99

[a] Orange values are weighted average of peel and pulp data